Adrenocorticotropic Hormone (ACTH) But Not Alpha-Melanocyte Stimulating Hormone (α-MSH) as a Mediator of Adrenalectomy Induced Hair Growth in Mink

To the Editor:

In the mink (*Mustela vison*), winter hair growth (anagen) usually begins in mid-September in response to reduced photoperiod, whereas summer anagen begins in late April as the photoperiod increases. We have demonstrated that bilateral adrenalectomy (ADX) during the resting (telogen) stage of either the summer or the winter hair growth cycle initiates anagen 4–5 wk earlier than controls (Rose and Sterner, 1992; Rose, 1995). The mechanism for ADX induced hair growth is unknown, although Paus *et al* (1994b) demonstrated a localized anagen response in the mouse 12 d after two intradermal injections of adrenocorticotropic hormone (ACTH). Because pituitary secretion of ACTH would be expected to increase following ADX, it is possible that ADX induced hair growth is mediated through this hormone; however, because blood ACTH levels fluctuate in response to many forms of stress, it seems unlikely that pituitary ACTH would function as a regulator of hair growth cycles under normal physiologic conditions. Nevertheless, ACTH, alpha-melanocyte stimulating hormone (α-MSH), and other proopiomelanocortin derived peptides and their receptors are synthesized by the skin (Slominski *et al*, 1992, 1996a; Wintzen and Gilchrest, 1996; Luger *et al*, 1997). In addition, Slominski *et al* (1992, 1996b) illustrated that expression of the proopiomelanocortin gene was higher during anagen than telogen in the skin of mice.

The objective of this study was to determine if ACTH and/or α-MSH would induce anagen in mink that were in summer telogen. On July 1 1997, eight adult female mink were housed in individual cages in a light tight room and exposed to a photoperiod approximating natural changes in day length. After shearing the fur over the lower half of the dorsal surface, four mink were injected intradermally in the right flank region with α-MSH (Peninsula Laboratories, Belmont, CA) at 5.0×10^{-5} M in 500 μl of phosphate buffered saline (PBS) on July 21, 23, and 25. On the same days, an additional four mink were injected intradermally in the left flank with ACTH (Sigma, St. Louis, MO) at the same concentration. The contralateral side of each mink was injected with PBS. Subsequently, the mink were observed daily for the appearance of blue pigmentation at the injection site as evidence of anagen onset.

On August 12, 18 d after the last injection, a dark blue spot was observed in all ACTH treated mink over the injection site only, whereas no α-MSH treated mink exhibited a change in pigmentation. On August 28 all ACTH treated mink exhibited hair growth averaging 4 mm over the injection site (**Fig 1*A***). None of the α-MSH treated mink exhibited hair growth over the injection site, although two animals did display hair growth over the spinal column (probably due to scraping the skin with shears), one of which had rather well developed guard hair growth over the entire sheared area (**Fig 1*B***). We inadvertently injected the ACTH treatment group on the right and left side with ACTH on July 23; however, as can be seen, the single injection of ACTH was clearly not sufficient to induce hair growth.

These findings suggest to us that ADX induced anagen in mink occurs as a result of elevated ACTH that acts directly on the skin. This most likely explains why ADX induces anagen during both summer and winter telogen. The fact that α-MSH did not induce hair growth or pigmentation suggests that this peptide is not involved in anagen initiation and/or that ACTH and α-MSH act through separate receptors in mink skin. The mechanism for ACTH induced anagen remains to be determined although it seems reasonable to ask: Does ACTH influence the metabolism of steroid hormones in the skin in a manner akin to that of the adrenal gland? The dermal papilla of the hair follicle (essential for anagen induction) and adrenal cortex are both derived from mesenchyme. Skin is a highly steroidogenic tissue, metabolizing androgens and estrogens, both of which have been shown to have profound effects on hair growth cycles. Moreover, Slominski *et al* (1996a) recently demonstrated that the gene for cytochrome P450c21, which produces 11-deoxycorticosterone and 11-deoxycorti-

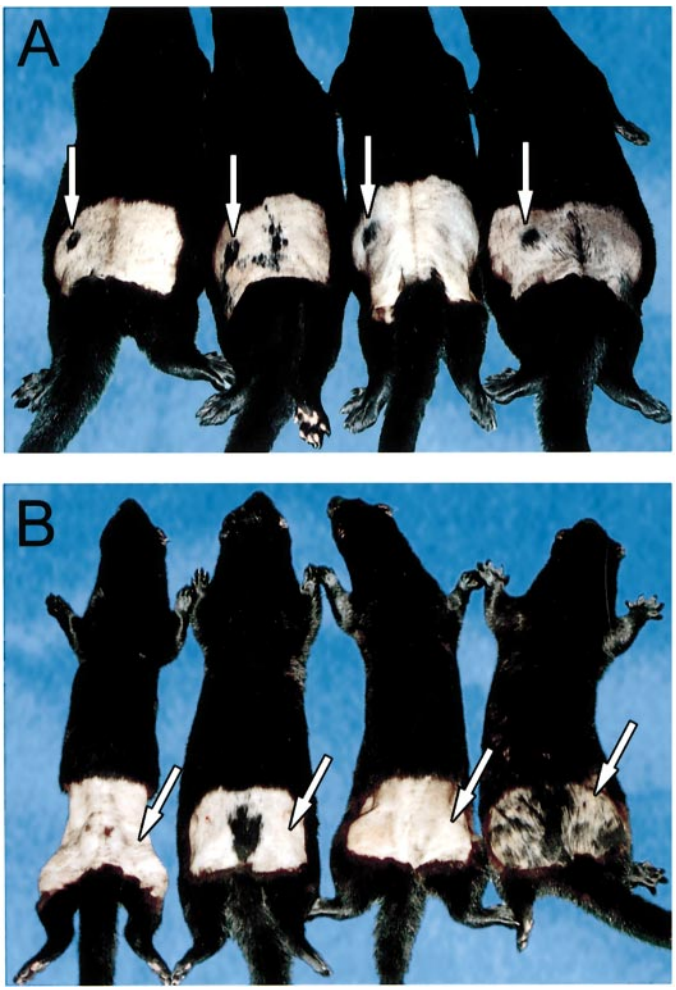

Figure 1. Anagen response to intradermal injections of ACTH or α-MSH. Mink were injected intradermally in the left flank with ACTH (*A*) or in the right flank with α-MSH (*B*) at 5.0×10^{-5} M in PBS once daily on July 21, 23, and 25 1997. The contralateral hip of each mink was injected with PBS only. The response pictured was on August 28, 34 d after the last injection.

sol (precursors to corticosterone and cortisol, respectively), was expressed in human skin. The possibility of glucocorticoid production in the skin coupled with the observations that topical corticoids block the development of anagen (Stenn *et al*, 1993), suppress proopiomelanocortin expression (Ermak and Slominski, 1997), and induce follicular regression (Paus *et al*, 1994a), should attract more intense research efforts in this area. Perhaps in species like the mink, which display photoperiodically controlled hair growth cycles, the onset of anagen occurs, in part, as a result of the seasonal production of ACTH and the subsequent metabolism of steroid hormones, both of which occur within the skin. Such a phenomenon might also contribute to the development of different pelage types such as dense winter fur and sparse summer fur as a result of differential activation of under hair type fibers. Adrenalectomized animals such as the mink should provide an excellent model with which to investigate the role of endogenous ACTH in hair growth cycles.

This work was supported in part by the American Mink Farmers Research Foundation (Corvallis, Oregon), the Canada Mink Breeders Association (Toronto, Canada), and a donation from American AGCO, Inc. (South St Paul, Minnesota). Mink were supplied by Mr. Lee Moyle of Heyburn, Idaho. I thank Mr. Jim Peck, Mr. Jerry Van Tassel, and Mr. Stan Burr for care of the mink. I especially appreciate the assistance of Mr. Brandon Ihli, Mr. Josh Billingsley, Mr. Chad Nichols, and Ms. Joy Mayhew in administering treatments. The mink were photographed by Dr. William Saul and Ms. Kelly Wood.

Jack Rose
Idaho State University, Department of Biological Sciences,
Pocatello, Idaho, U.S.A.

REFERENCES

Ermak G, Slominski A: Production of POMC, CRH-R1, MC1, and MC2 receptor mRNA and expression of tyrosinase gene in relation to hair cycle and dexamethasone treatment in the C57BL/6 mouse. *J Invest Dermatol* 108:160–165, 1997

Luger TA, Scholzen T, Grabbe S: The role of α-melanocyte-stimulating hormone in cutaneous biology. *J Invest Dermatol Symp Proc* 2:87–93, 1997

Paus R, Handjiski B, Czarnetzki BM, Eichmüller S: A murine model for inducing and manipulating hair follicle regression (catagen): Effects of dexamethasone and cyclosporin A. *J Invest Dermatol* 103:143–147, 1994a

Paus R, Maurer M, Slominski A, Czarnetzki BM: Mast cell involvement in murine hair growth. *Dev Biol* 163:230–240, 1994b

Rose J: Bilateral adrenalectomy induces early onset of summer fur growth in mink *(Mustela vison)*. *Comp Biochem Physiol* 111C:243–247, 1995

Rose J, Sterner M: The role of the adrenal glands in regulating onset of winter fur growth in mink (*Mustela vison*). *J Exper Zool* 262:469–473, 1992

Slominski A, Paus R, Mazurkiewicz J: Proopiomelanocortin expression in the skin during induced hair growth in mice. *Experientia* 48:50–54, 1992

Slominski A, Ermak G, Mihm M: ACTH receptor, CYP11A1, CYP17 and CYP21A2 genes are expressed in skin. *J Clin Endocrinol Metab* 81:2746–2749, 1996a

Slominski A, Ermak G, Hwang J, Mazurkiewicz J, Corliss D, Eastman A: The expression of proopiomelanocortin (POMC) and of corticotropin releasing hormone receptor (CRH-R) genes in mouse skin. *Biochimica et Biophysica Acta* 1289:247–251, 1996b

Stenn KS, Paus R, Dutton T, Sarba B: Glucocorticoid effect on hair growth initiation: A reconsideration. *Skin Pharmacol* 6:125–134, 1993

Wintzen M, Gilchrest BA: Proopiomelanocortin, its derived peptides, and the skin. *J Invest Dermatol* 106:3–10, 1996

Inhibition of Chemotherapy-Induced Keratinocyte Apoptosis *In Vivo* by an Interleukin-15-IgG Fusion Protein

To the Editor:

The control of keratinocyte apoptosis *in vivo* under physiologic and pathologic conditions is still quite unclear. During spontaneous or induced regression of the hair follicle, remodelling is largely brought about by the massive, yet highly controlled apoptosis of keratinocytes in the proximal follicle epithelium (Weedon and Strutton, 1981). The hair follicle therefore offers an ideal, yet often ignored model system for studying the control of keratinocyte apoptosis *in situ* (Paus *et al*, 1993; Lindner *et al*, 1997).

Among the secreted signals that could serve to downregulate keratinocyte apoptosis *in situ* (Polakowska and Haake, 1994; White, 1996), interleukin-15 (IL-15) has recently surfaced as an especially intriguing candidate because it suppresses multisystem apoptosis induced by anti-Fas antibodies in mice as well as anti-Fas and dexamethasone-induced apoptosis in T and B cells *in vitro* (Bulfone-Paus *et al*, 1997). Furthermore, anti-Fas-induced lethal multisystem apoptosis in mice is suppressed by an IL-15-IgG2b fusion protein (FP), resulting in the survival of all IL-15 FP treated mice (Bulfone-Paus *et al*, 1997). These data raise the possibility that IL-15 may also inhibit keratinocyte apoptosis.

The massive upregulation of keratinocyte apoptosis that is associated with chemotherapy-induced follicle dystrophy and alopecia (Lindner *et al*, 1997) is well suited to detect apoptosis-suppressing effects of candidate agents. Using a mouse model for the study of chemotherapy-induced alopecia and follicle dystrophy (Paus *et al*, 1994), we have addressed the question of whether cyclophosphamide (CYP)-induced apoptosis of keratinocytes in the hair bulb of anagen VI follicles *in vivo* is down-modulated by prior administration of IL-15-IgG2b FP (for details see Bulfone-Paus *et al*, 1997). As controls, either CYP alone or CYP and an IL-2-IgG2b FP (Kunzendorf *et al*, 1996) were injected, because keratinocytes do not express IL-2 receptors.

Twenty-four hours after the intraperitoneal injection of 120 mg CYP per kg into C57BL/6 mice with all back skin hair follicles in early anagen VI (Paus *et al*, 1994), with or without coadministration of 100 μg IL-15-IgG2b FP (Bulfone-Paus *et al*, 1997) or IL-2-IgG2b FP (Kunzendorf *et al*, 1996), back skin cryosections were prepared and stained for apoptotic cells, using a fluorescent TUNEL method with a commercially available *in situ* end-labeling kit (Apoptag^R, Oncor, Gaithersburg, MD), combined with counterstaining by Hoechst 33342 (Lindner *et al*, 1997).

The number of TUNEL$^+$ keratinocytes in the proximal hair bulb of anagen VI follicles with the characteristic Hoechst 33342 morphology of apoptotic cells was quantitated and compared between CYP, CYP + IL-15-IgG2b, and CYP + IL-2-IgG2b FP treated mice.

A single administration of the IL-15-IgG2b FP (100 μg), but not of the control IL-2-IgG2b FP, inhibited CYP-induced keratinocyte apoptosis in murine anagen hair bulbs *in vivo* (**Figs 1**, **2**). This inhibitory effect was discrete (reduction by ≈24%), but statistically significant ($p < 0.001$), when the total number of apoptotic cells in CYP– *versus* CYP + IL-15-IgG FP treated hair follicles was compared by quantitative histomorphometry (**Fig 2**).

Whereas the mechanisms underlying this apoptosis-inhibitory property of an IL-15-IgG FP remain to be elucidated, these data demonstrate that, in the current model, stimulation of the IL-15

Reprint requests to: Dr. Ralf Paus, Cherité, Department of Dermatology, Humboldt University, Schumannstr. 20/21, D-10117 Berlin, Germany.

Abbreviations: CYP, cyclophosphamide; FP, fusion protein.